THE VALUE OF OPSONIN DETERMINATIONS IN THE DISCOVERY OF TYPHOID CARRIERS.*

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THE comparatively recent discovery of so-called bacillus carriers (typhoid and paratyphoid) has lent additional interest to the question of immunity toward typhoid and paratyphoid bacilli, for it is evident that there must be some modification of the bacillus itself or of the defensive properties of the carrier's tissues to make possible the prolonged harboring of these pathogenic organisms without the occurrence of a general infection. The literature on typhoid and paratyphoid carriers is already quite extensive and I shall refer to it only as it bears on this point of the carrier's immunity to his own bacillus.

It is natural that observations on the presence of agglutinins in the carrier's serum should be far more numerous than those on the other antibodies. As a usual thing in the literature we find that the blood of the carrier has been tested for agglutination of the typhoid bacillus, either the carrier's own strain or a stock culture, and in the majority of instances agglutination has occurred tho seldom in high dilution. Förster and Kayser[1] state that the serum of chronic carriers usually contains agglutinin for the corresponding bacillus. Lentz[2] obtained agglutination in 10 of his 11 carriers in dilution of 1:50, more rarely 1:100.

That the persistence of agglutinin for long periods after recovery is probably due to the persistence of the bacilli in the body is held by Kutcher[3] and he would also explain the occasional occurrence of agglutinin for the typhoid bacillus in cases of icterus, meningitis, sepsis, etc., by assuming that such persons were in fact typhoid carriers. Steinberg,[4] who found that 15 out of 22 icteric patients agglutinated typhoid bacilli in dilution of at least 1:40, attributed these results to group agglutination caused by an infection with colon bacilli or with one of the proteus group, but Quènu[5] believes that the explanation lies in a primary infection of the gall-bladder with typhoid bacilli and F. Ehrlich[6] has reported two cases of such primary infection under the name of "biliary typhoid."[7]

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[1] *Münch. med. Wchnschr.*, 1905, 52, p. 1471. [2] *Klin. Jahrb.*, 1905, 14, p. 475.

[3] Kolle und Wassermann, *Handbuch*, Ergbd. 1, 1907, pp. 260, 655.

[4] *Münch. med. Wchnschr.*, 1904, 51, p. 469.

[5] *Rev. de Chirurg.*, 1908, 38, p. 571. [6] *Deut. med. Wchnschr.*, 1906, 32, p. 1704.

[7] A case of paratyphoid infection of this sort came under my observation in the person of a young Greek, with jaundice, fever, clay stools, etc. The patient had never had typhoid fever and this was the first attack of the present character. His index to paratyphoid bacillus B, was 0.3, 1.2, 4.1, and 5.0 the examination extending over a period of nine days. Paratyphoid bacillus B was isolated from his stools and agglutinated by his serum in dilution of 1:80.

On the other hand it has often been found that the typhoid bacillus may be present in large numbers in the body of a carrier and yet the serum show no agglutinating power above that which may be found in normal serum. Dörr[1] claims, on the basis of animal experiments, that if typhoid bacilli are present in the intestinal tract and gall-bladder only, the agglutinating power of the serum will not be above 1:22 and that a higher agglutinating power is found only when the bacilli have passed into the blood stream. Even this, however, may occur without causing any production of agglutinins, as was shown in Busse's four remarkable cases. Busse[2] isolated typhoid bacilli in pure culture from the blood of four non-typhoid patients but found that the serum of all four was without agglutinating power. His theory was that these persons were undetected typhoid carriers and that ulcerative or desquamative lesions in the intestinal tract had allowed the typhoid bacilli to pass into the blood, where their presence seemed to cause no changes. Three of the cases were in the last stages of tuberculosis, with intestinal ulcers, the other was a pneumonia case who recovered.

On the whole, then, agglutinins for typhoid bacilli are not always to be found in the blood of typhoid or paratyphoid carriers. Kamm[3] and Gaetgens[4] consider this test of very little diagnostic value for the detection of bacillus carriers.

When it comes to the question of an increased bactericidal power in the blood of carriers there are but few recorded observations and those we have are not very conclusive. A slight increase of bactericidal power was noted in Ledingham's[5] cases. Kutcher[6] also reports a high bactericidal power in the serum of one carrier whom he examined.

There is one published observation on complement deviation. Schöne[7] found complement-deviating substances in the blood of three typhoid carriers and claims that this reaction can be obtained at least as constantly as agglutination. His three carriers gave the following, only partly satisfactory, results:

		Aggl. 1:50	Complement Deviation
Case 1.	Typhoid fever 10 years ago	−	+
Case 2.	In contact with typhoid patient 2 years ago	−	−
Case 3.	Typhoid fever 12 weeks ago	+	++

The failure on the part of the great majority of observers to find any decided increase in protective substances in the serum of typhoid and paratyphoid carriers has led to a general adoption of the often quoted theory of Wassermann and Citron[8] which attributes the immunity of carriers to a local biologic change in the tissue cells as a result of prolonged contact with the bacteria in question. As numerous observers have shown the seat of infection in typhoid and paratyphoid carriers to be the biliary tract it is the lining cells of these structures which are responsible for the defense of the body. This local cellular resistance to bacteria, which may persist for a lifetime, is not due to the continued production of antibodies, for none can be demonstrated in the blood serum. It is rather an obscure biologic change in the cells, rendering them

[1] *Centralbl. f. Bakt.*, Abt. 1, Orig., 1905, 39, p. 624.

[2] *Münch. med. Wchnschr.*, 1908, 55, p. 1113.

[3] *Ibid.*, 1909, 56, p. 1011.

[4] *Deut. med. Wchnschr.*, 1909, 35, p. 1337.

[5] *Brit. Med. Jour.*, 1908, 2, p. 1173.

[6] Kolle und Wassermann, *Handbuch*, Ergbd. 1, 1907, p. 260.

[7] *Münch. med. Wchnschr.*, 1908, 55, p. 1065.

[8] *Deut. med. Wchnschr.*, 1905, 31, p. 573.

insusceptible to the attacks of the invading germs. To this acquired cellular immunity of Wassermann and Citron, Lübke[1] would add an acquired immunity on the part of the carrier's own strain, "eine erworbene Serumfestigkeit." He found that in experimentally injected animals the bacilli would persist longest in those very tissues (spleen, marrow) the extracts from which were richest in antibodies. Therefore it is impossible to gauge the resistance of an organism by the bactericidal power of the body fluids or cells, for the presence of these antibodies calls forth the production of corresponding defensive substances in the body of the bacillus. Thus in the case of bacillus carriers there is established a sort of armed truce between the antibodies of the carrier and the bacterium.

It is only in the very recent literature that we find any mention of opsonin in the blood of typhoid and paratyphoid carriers. Ledingham[2] in 1908 examined the serum of two typhoid carriers with regard to their opsonic index, using inactivated and complemented serum, and found the index very high both to the carrier's own strain and to that of the other carrier. A few weeks ago Gaetgens[3] published the results of his study of the opsonic index in 12 typhoid convalescents, who had not become carriers, and in 16 chronic typhoid carriers and 2 chronic paratyphoid carriers. He found that typhoid convalescents who did not become carriers exhibited an opsonic index above normal only for a short period, three or four months at the outside, while typhoid carriers had a persistently high index, irrespective of the lapse of time since recovery from typhoid fever or even in the absence of any history of typhoid fever. As all but one of these carriers had an index above 1.4, the average being 2.8 (Gaetgens uses unheated serum and his counts are therefore not as high as Ledingham's), and as 25 per cent had failed to agglutinate in dilutions higher than 1:50, Gaetgens considers the opsonic index of more value in the detection of carriers than the agglutination test. His two paratyphoid carriers, on the other hand, gave only normal indices, a result which he thinks may be due to the fact that the laboratory strain used had lost its virulence, but he believes that possibly this may prove to be a distinction between typhoid and paratyphoid carriers.

One cannot help suspecting that the case recently reported by Aaser[4] may have been a typhoid carrier. The man had had typhoid fever in 1898, his serum had lost all agglutinative power six months after his recovery, but when his opsonic index to the typhoid bacillus was taken in 1907, it was found to be very high, 6.0 and 7.0.

It is interesting to note the increasing number of observations in the literature which tend to show that the immunity on the part of bacillus carriers is, after all, only partial in most cases and liable to complete breakdown. The presence in the gall bladder and passages of typhoid or paratyphoid bacilli leads frequently to a chronic cholecystitis with or without the formation of stones, and recurring attacks of fever and jaundice are not rare in the history of carriers. Lentz[5] says that there is a direct relation between slow recovery with lasting ill-health and a persistence of the germs in the body. Dean's[6] and Schiller's[7] cases apparently confirm this statement. In

[1] *Münch. med. Wchnschr.*, 1909, 56, p. 57.
[2] *Brit. Med. Jour.*, 1908, 2, p. 1173.
[3] *Deut. med. Wchnschr.*, 1909, 35, p. 1337.
[4] *Jahresb. f. Immunitätsforschung*, 1908, 3, p. 86.
[5] *Klin. Jahrb.*, 1905, 14, p. 475.
[6] *Brit. Med. Jour.*, 1907, 50, p. 562.
[7] *Wien. med. Wchnschr.*, 1908, p. 12.

other cases the bacillus lodges in different parts of the body, setting up a suppurative process, many months after recovery from the original sickness or even without any history of typhoid fever. Bauer[1] has collected eight instances of suppurative chondritis due to the typhoid bacillus, occurring from two months to two years after recovery from typhoid fever. Kamm[2] reported two similar cases, one of whom had no history of typhoid fever.

Two striking instances of complete loss of immunity in old typhoid carriers have been described by Levy and Kayser[3] and by Grimme.[4] In both of them there was a history of typhoid fever, followed by persistence of the bacilli in the biliary tract, a chronic cholecystitis with stones (in both cases the stones yielded cultures of typhoid bacilli), and finally a re-autoinfection from the gall bladder, causing fatal typhoid bacteriemia. Another, but not fatal case, of the same general infection occurring in a carrier with cholecystitis is reported by F. Ehrlich.[5]

The following study was undertaken with the hope of adding to the small number of observations now available on the antibodies in the serum of typhoid and paratyphoid carriers, especially the opsonin, but in the course of the work other questions arose which will also be discussed briefly.

As no known typhoid carriers were available, I chose for my search a class of patients who would supposedly yield a larger proportion of carriers than any other, namely, persons with chronic cholecystitis. It is unnecessary to review the extensive literature which has shown that chronic inflammatory lesions of the biliary tract are in many instances caused by typhoid or paratyphoid bacilli. More recently the reports on typhoid carriers have laid stress on the frequent presence of gall-stones in the carriers and upon the fact that the proportion of multiparous women among bacillus carriers is similar to the proportion of multiparous women suffering from gall-stones[6] (Förster and Kayser;[7] Muller[8]).

Of the 24 cases selected, all but one were patients with various lesions of the gall tract. Twenty were middle-aged married women, one was an unmarried women of 50 years, and one a man of 28 years. The twenty-fourth was a woman in normal health who had a history

[1] Inaugural Diss., Rostock, 1894.

[2] *Münch. med. Wchnschr.*, 1909, 56, p. 1011.

[3] *Arb. a. d. kais. Gesundh.*, 1907, 25, p. 254.

[4] *Münch. med. Wchnschr.*, 1907, 54, p. 1822.

[5] *Deut. med. Wchnschr.*, 1906, 32, p. 1704.

[6] A more conservative stand on the question of the relation between typhoid infection and cholecystitis is found in a recent article by Eug. Fraenkel, *Mitth. a. d. Grenzgebiet. d. Med. u. Chi.*, 1909, 20, p. 898.

[7] *Münch. med. Wchnschr.*, 1905, 52, p. 1471.

[8] *Am. Jour. Med. Sci.*, 1908, 126, p. 314.

of both typhoid and paratyphoid fever. Five had had typhoid fever, but only one of them as recently as four years ago. This last case traced her present trouble directly to an unusually severe attack of typhoid fever. A brief résumé of the important facts in the histories of these patients is given in Table 1 and I shall not go into details now except to state that at the time the examination was made four of the patients were suffering from an acute attack of colic, jaundice, fever, clay stools, etc., and gave a history of similar symptoms in the past. The other 20 were at the time free from acute symptoms.

The principal results of the study of these cases would come under the following heads: A. The large proportion of carriers among cases of chronic cholecystitis. B. The incompleteness and instability of the carrier's immunity to his own bacillus. C. The abnormal opsonic index to typhoid or paratyphoid bacilli in the serum of carriers. D. The close relation between typhoid and paratyphoid bacilli.

THE LARGE PROPORTION OF CARRIERS AMONG CASES OF CHRONIC CHOLECYSTITIS.

The following examinations were made in these cases:

1. The patient's stool, urine, and, in operative cases, bile or pus, were examined for the presence of typhoid or paratyphoid bacilli. Malachite green agar plates (containing malachite green 1:2,000 or 1:2,500) were spread with the material in question, allowed to stand for 24 hours at 37° C., the colonies then washed off with salt solution and spread upon plates filled with Endo and with Drigalski-Conradi medium. This malachite green agar had been found to inhibit the growth of three laboratory strains of colon bacilli, while control strains of typhoid and paratyphoid bacilli grew well upon it.

2. The patient's serum was used for agglutination tests (macroscopic) with a stock strain of typhoid bacillus, one of colon bacillus, and two strains of paratyphoid bacilli, type B and type A. The strain of type B was kindly furnished by the Bacteriological Laboratory of the University of Chicago, together with four other strains which were frequently used for comparison in cultural and agglutination tests.

3. The opsonic index of these four organisms was also estimated, the patient's serum and the control serum being heated first to 55°–60° C. for 20 minutes. As this exposure is enough to inactivate the lysin in both normal (see Klien;[1] Clark and Simonds[2]) and immune serum, the difference in the action of the thermostable opsonic substance in the two becomes much more striking than in unheated specimens. The same strain of typhoid bacilli and the same strains of paratyphoid bacilli, A and B, were used in all the tests. None of them was spontaneously phagocytable in the suspensions employed.

[1] *Bull. Johns Hopkins Hosp.*, 1907, 18, p. 245.

[2] *Jour. Infect. Dis.*, 1908, 5, p. 2.

4. In patients who had acute symptoms a bacteriological examination of the blood was made by allowing from 2 to 5 c.c of sterile blood to run into a test tube containing 5 to 10 c.c. of sterile ox bile. After 24 hours' incubation the culture was spread on Drigalski-Conradi plates.

5. Bacteriolytic experiments were made with the patient's blood in all but one of the positive cases, the method described by Neufeld and Hüne[1] being followed.

The typhoid bacillus was identified culturally by the production of typical colonies on agar plates and on Endo and Drigalski-Conradi plates, by the growth in milk, litmus whey, neutral red agar, and on potato. Agglutination tests were made with the serum of rabbits injected with a laboratory strain of typhoid bacilli and, as a final test, Castellani's absorption method[2] was used.

In identifying the paratyphoid bacilli less stress was laid on cultural characteristics than upon agglutination with sera of rabbits which had been injected with control strains of paratyphoid bacilli. Rabbits were injected with the same strains of paratyphoid A and B which were used in the agglutination and opsonic tests. These immune sera showed group agglutination for the control strains of typhoid, paratyphoid, and colon bacilli. The serum of the rabbit injected with paratyphoid A agglutinated one stock strain of paratyphoid B and agglutinated feebly three colon strains. That of rabbit paratyphoid B did not agglutinate paratyphoid A or the strains of colon bacilli, but did agglutinate two strains of typhoid bacilli almost as strongly as it did the strain of paratyphoid B used in immunization. It failed to agglutinate three stock strains of paratyphoid B.

In the course of the examination of the 24 cholecystitis cases, 12 organisms were isolated two of which proved to be typhoid bacilli, typical in all respects. Three belonged to the colon group but were not typical, in that they produced but very little gas in glucose media, grew well upon the malachite green plates, and formed pink colonies on Endo plates instead of the deep red ones with metallic luster which are typical of colon bacilli. They also agglutinated strongly with the serum of the rabbit immunized against paratyphoid A. The remaining 7 organisms were apparently paratyphoid bacilli, but it would be difficult to classify all of them on the ground of cultural characteristics alone. Three corresponded to the usual description

[1] *Arb. a. d. kais. Gesundh.*, 1907, 25, p. 164.

[2] *Ztschr. f. Hyg.*, 1902, 40, p. 1.

of type B except that they formed a typhoid-like growth on potato. They also agglutinated with type B immune serum and I have therefore classified them under that head. One agglutinated with the same serum and formed a colon-like growth on potato—belonging also to type B. The fifth belonged culturally to this type, but agglutinated with serum type A. The sixth agglutinated only with the patient's serum and culturally resembled type A, while the seventh was apparently a typical member of type A both culturally and by serum tests. Naturally, all the strains which resembled the colon bacillus culturally and which failed to agglutinate with immune rabbit serum or with the patient's serum were rejected.

Of the 24 cases examined only those were pronounced to be carriers who fulfilled the following requirements:

1. The presence in the patient's body of a more or less motile, gram-negative bacillus, agglutinated by the serum of rabbits immunized against typhoid or paratyphoid bacilli, or by the patient's serum.

2. The presence in the patient's serum of an abnormal opsonic index for stock strains of the typhoid bacillus or paratyphoid bacillus B or A, and of agglutinin or opsonin for the patient's own bacillus.

Seven of the 24 patients presented these features, five being paratyphoid carriers, one a typhoid carrier, and one harboring both typhoid and paratyphoid bacilli. This represents a proportion of 29 in a hundred, and corresponds to the results obtained by Blumenthal,[1] who examined 17 cases of cholecystitis and found that four harbored typhoid bacilli and one paratyphoid type A, making a proportion of 29 in a hundred.

Three of my seven positive cases had a history of typhoid fever, two of the seventeen negative cases had such a history. It is interesting to note that neither typhoid carrier had ever, so far as could be discovered, been the cause of infection in others. One was a woman of wealth who did not handle the food of the household, the other prepared the food for her own family but had very cleanly habits.

IMMUNITY OF CARRIER TO HIS OWN BACILLUS.

In only one of the seven carriers was there complete immunity to the infecting agent, altho the histories of these cases showed that

[1] *Mediz. Klinik*, 1905.

the bacilli had been harbored for periods running from two months to eight years. Three gave histories of repeated attacks of biliary colic, and at the time of examination were recovering from operative removal of gall-stones. One was waiting operation and during this time had two attacks of fever and jaundice. Another was recovering from an operation for abscess of the liver and the sixth was suffering from his second attack of acute catarrhal jaundice. The one case who was apparently quite normal gave a history of typhoid fever 12 years ago and of paratyphoid fever (clinically diagnosed) four months before. She was one of the paratyphoid carriers.

Reference has been made to the three cases of typhoid bacteriemia reported by Levy and Kayser, by Grimm, and by Fr. Ehrlich. One similar case is included in my series, that of a paratyphoid carrier who developed a general bacteriemia following an operation for the removal of gall-stones and ending in recovery. This woman, Case No. 5, was a patient of Dr. C. H. McKenna[1] who has already published a clinical report of the case.

The important features from my point of view were the following: a history of an attack of cholecystitis dating back more than two years; a second attack during which an operation was performed, this being apparently the occasion for the escape of paratyphoid bacilli type A into the blood stream.[2] The patient's immunity to her bacillus was evidently lowered at the time of the attack and the operation caused a still greater loss of resistance. As will be seen later, it was possible in this case to show a loss of opsonin at the time of the bacteriemia and a very marked increase as convalescence set in.

ON THE PRESENCE OF ANTIBODIES IN THE SERUM OF TYPHOID AND PARATYPHOID CARRIERS.

Agglutinins.—Gaetgens rejects all sera which agglutinate in dilution not higher than 1:50. Lentz accepts 1:50 as positive. Dörr found that the serum of rabbits with experimental typhoid cholecystitis agglutinated in dilution no higher than 1:22. Bieberstein, reviewing the literature on agglutination in typhoid fever, found a decided difference of opinion as to what dilution of serum should

[1] *Jour. Am. Med. Assoc.*, 1909, 1, p. 239.

[2] This case is similar in all essentials to that reported by Libman, *Jour Med. Res.*, 1902, 3, p. 168..

be regarded as the limit beyond which a positive diagnosis should be made, the figures running from 1:20 to 1:50. He puts it at about 1:30, as he has found only 2 per cent of normal sera agglutinating at this point. Three of my five paratyphoid carriers agglutinated a stock strain or their own strain of paratyphoid or both in dilution of 1:50 or higher. Both typhoid carriers agglutinated typhoid bacilli, stock strain and homologous strain, in dilutions higher than 1:50, so that five out of seven carriers, or 71 per cent, gave positive results with the agglutination test. The case of proved mixt infection, the typhoid and paratyphoid carrier, agglutinated both organisms in dilutions of 1:80 and 1:100. Group agglutination was found in Case 4 whose serum agglutinated typhoid as well as paratyphoid bacilli without any other evidence of mixt infection, i.e., without the discovery of typhoid bacilli in the patient's body and without a high opsonic index to the stock strain which was agglutinated.

Opsonins.—Table 1 gives the agglutinations and opsonic indices in the eight carriers. This table shows that all seven carriers had an abnormal opsonic index for their own strain or for some stock strain of typhoid or paratyphoid bacilli. In three instances, Cases 4, 5, and 7, the paratyphoid bacilli isolated from the pus, blood, and urine respectively were at first non-phagocytable, but the patient's indices were found to be abnormal to stock strains of paratyphoid bacilli and later on to their own strains. The opsonic index therefore proved positive in 100 per cent of all carriers as against the 71 per cent of positive agglutination tests. These results correspond with those of Gaetgens quoted above.

Among the non-carriers were two, both former typhoid cases, who agglutinated typhoid bacilli in low dilution, 1:30 and 1:40. Another agglutinated paratyphoid A, 1:30. No decidedly high opsonic index was found among the non-carriers altho occasionally an index from 2.0 to 2.7 would be found in a serum which was usually normal. This was not regarded as significant in the absence of any other positive findings, for it could easily be explained on the ground of group opsonic action in a case of infection by one of the colon group. The two cases of mixt infection, Cases 1 and 2, had high indices for typhoid bacilli and for their own strains of colon bacilli, atypical, and Case 1 for paratyphoid B as well. Here opsonin and agglutinin corresponded.

Cases 4, 5, 6, and 7 had an abnormal index only for one organism, namely that corresponding to the strain later isolated. No group opsonic action could be shown in these cases. On the other hand, Case 3 had a high index to typhoid as well as to paratyphoid bacilli, altho she could not be shown to harbor any but the last-named organism. The same thing was true of Case 8, whom I have inserted with the others in the table because, tho not a carrier, the findings in her case corresponded so closely with those in Case 3. She too had a

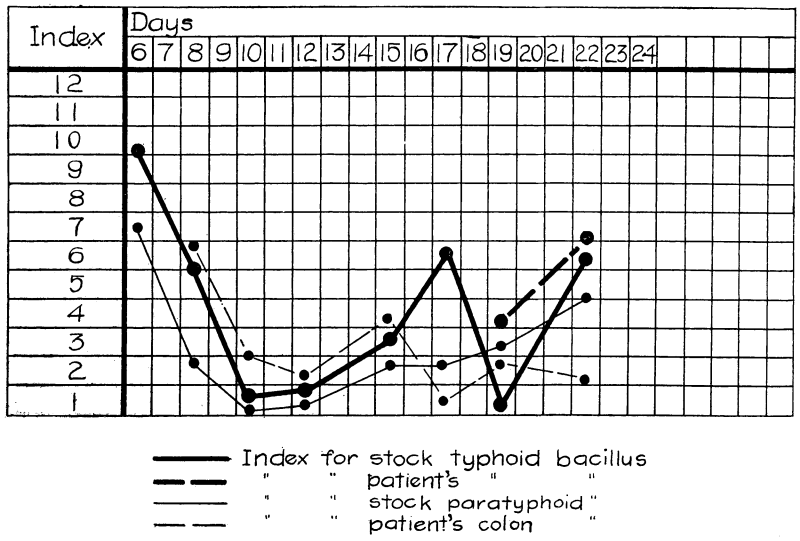

CHART 1.—Opsonin index of Case 2. Acute typhoid cholecystitis.

high index to typhoid bacilli as well as to her own strain of paratyphoid type B, but no typhoid bacilli were ever found in the course of repeated examinations of her stools. These anomalous opsonic indices will be discussed later in their bearing upon the question of cross opsonins, but I mention them here to show that while the opsonic test proved positive in all my carriers, it did not always serve to indicate whether the case was a typhoid or a paratyphoid carrier or both.

A study of the charts made from cases with acute symptoms shows that the amount of opsonin present fluctuated according to the increase or subsidence of the disturbance. Case 5 had a low index shortly after her operation, at the time when the blood contained

TABLE 1.

AGGLUTINATION AND OPSONIC INDEX OF CARRIER

No.	SEX AND AGE	TYPHOID FEVER	HOSPITAL	CONDITION	BACILLUS FOUND IN	BACILLI ISO
1. Typhoid and paratyphoid carrier	Married woman, 45 yrs.	4 years before	Presbyterian Hospital Dr. Bevan	Cholecystitis 4 years Operation: adhesions, constriction of cystic duct	Urine Bile Stool	Colon bacillus Paratyphoid b type B Typhoid bacil
2. Typhoid carrier	Married woman, 50 yrs.	No	Presbyterian Hospital Dr. Billings and Dr. Bevan	Cholecystitis 4 years Acute attack Operation: Stones	Urine Bile Stool	Colon bacillus Typhoid bacil
3. Paratyphoid carrier	Unmarried woman, 34 yrs.	12 years before. Paratyphoid fever 4 months before	West Side Hospital Dr. Van Hoosen	Normal	Stool	Paratyphoid b type B
4. Paratyphoid carrier	Married woman, 46 yrs.	No	Presbyterian Hospital Dr. Herrick and Dr. Bevan	Abscess of liver Operation	Pus from abscess	Paratyphoid b type B
5. Paratyphoid carrier	Unmarried woman, 40 yrs.	No	St. Joseph's Hospital Dr. McKenna	Second acute attack First attack 2½ years ago Operation: Stones Bacteriemia	Blood	Paratyphoid b type A
6. Paratyphoid carrier	Man, 28 yrs.	5 years before	Presbyterian Hospital Dr. Billings	Second acute attack First attack 2 months ago	Stool	Paratyphoid b type B
7. Paratyphoid carrier	Married woman, 32 yrs.	No	Cook County Hospital	Cholecystitis 8 years Operation: Stones	Urine	Culturally like phoid bacill B. Aggluti with type A
8. Not shown to be a carrier	Unmarried woman, 33 yrs.	Had nursed typhoid cases	Presbyterian Hospital Dr. Herrick	Paratyphoid fever	Urine Stool	Paratyphoid b type B Colon bacillus

* S.= stock: P.= patient's strain.

TABLE 1.

GGLUTINATION AND OPSONIC INDEX OF CARRIERS.

CONDITION	BACILLUS FOUND IN	BACILLI ISOLATED	AGGLUTINATION				OPSONIC INDEX			
			Typhoid Bacillus	Paratyphoid Bacillus A	Paratyphoid Bacillus B	Colon Bacillus	Typhoid Bacillus	Paratyphoid Bacillus A	Paratyphoid Bacillus B	Colon Bacillus
olecystitis 4 years Operation: adhesions, constriction of cystic duct	Urine Bile Stool	Colon bacillus (atypical) Paratyphoid bacillus, type B Typhoid bacillus	+1:200 S. and P.*	.....	+1:300 P.	+1:150 P.	6.0:16.0 S. and P.	.....	3.0:8.0 S. and P.	2.2:4.6 P.
olecystitis 4 years Acute attack Operation: Stones	Urine Bile Stool	Colon bacillus (atypical) Typhoid bacillus	+1:100 S. and P.	.....	.....	+1:80 P.	1.1:9.8 S. and P.	.....	.....	1.1:6.8 P.
ormal	Stool	Paratyphoid bacillus, type B	+1:30 S.	.....	+1:30 P.	.....	5.2:21.0 S.	.....	3.3:15.0 P. and S.	
bscess of liver Operation	Pus from abscess	Paratyphoid bacillus, type B	+1:50 S.	.....	+1:150 S. and P.	.....	1.9:2.7	.....	0.53:12.5 P. and S.	
cond acute attack rst attack 2½ years ago Operation: Stones cteriemia	Blood	Paratyphoid bacillus, type A	.....	+1:300 P. and S.	.....	.....	.....	0.6:31.0 P. and S.		
cond acute attack rst attack 2 months ago	Stool	Paratyphoid bacillus, type B	.....	.....	+1:80 S.	.....	.....	.....	1.4:10.0 P. and S.	
olecystitis 8 years Operation: Stones	Urine	Culturally like paratyphoid bacillus, type B. Agglutinated with type A serum	.....	.....	.....	.....	.....	3.5:6.0 S.		
ratyphoid fever	Urine Stool	Paratyphoid bacillus, type B Colon bacillus (atypical)	+1:40 S.	.....	+1:200 P. and S.	+1:40 S.	4.0:22.0 S.	.....	1.9:14.0 P. and S.	2.0:10.0 P.

paratyphoid bacilli. It rose gradually and reached 31.0 during convalescence, then sank to 5.5, and after complete recovery, seven months after her operation, it stood at 11.0. Case 4 had an index of 0.53 at the time when her temperature was high and there was a profuse discharge from the liver abscess. Before leaving the hospital

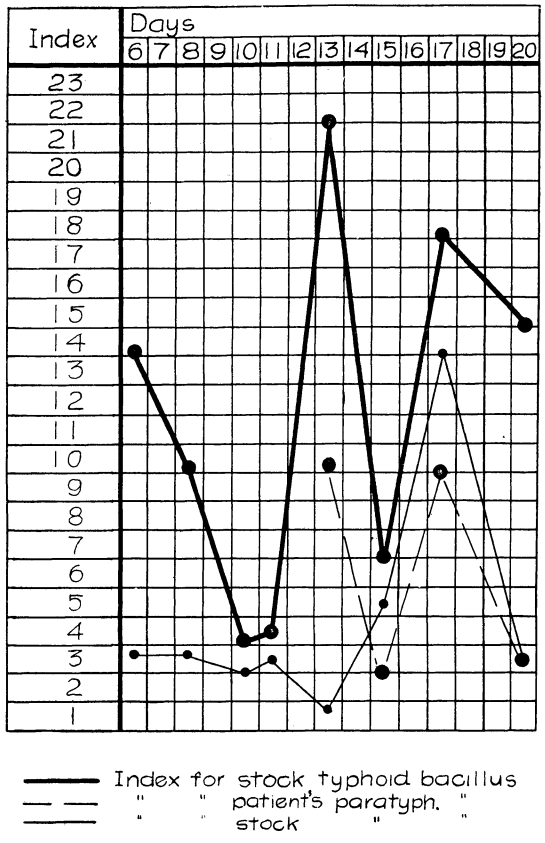

CHART 2.—Opsonin index of Case 8. Paratyphoid fever.

her index had risen to 12.5. The charts of Cases 2 and 8, which are reproduced, show how the index varied with the course of the disease. The chronic cases on the other hand, without acute symtoms, had a fairly steady, high index, rising not much over 8.0, seldom falling below 3.5 and never below normal. When double infection existed, and in those cases in which, without demonstrable double

infection, high indices for two different organisms were found, the indices to the different organisms were strikingly similar (Charts 1 and 2).

It was not possible in most of these cases to show a diminution in the carrier's opsonin toward her own strain as compared to stock strains nor greater resistance to phagocytosis on the part of the carrier's strain as compared to stock strains. Three times only, in Cases 4, 5, and 7, the strain of paratyphoid isolated from pus, blood, and urine respectively exhibited a marked resistance to phagocytosis in the presence both of normal and of the patient's serum. This characteristic was, however, soon lost (except by the bacillus of Case 7) and it may be that the stage of resistance in the other strains had already passed when the organisms were tested, for usually several days of cultivation on artificial media had elapsed before it was possible to decide on the character of the strain and make the opsonic tests.

Bacteriolysin.—Experiments to determine the bactericidal power of the carrier's serum for laboratory strains, and for the individual strain isolated, were made in Cases 1, 2, 3, 4, 5, and 8. Case 7 was tested only with laboratory strains. The carrier's serum and the control normal serum were inactivated by heat and complemented with normal rabbit serum, following the method advised by Neufeld and Hüne.[1] In no case was there any striking or constant difference between the carrier's serum and the normal serum, not even in the case of the patient with paratyphoid bacteriemia. Nor was it possible to show an increase in resistance on the part of the carrier's strain as compared with the control strain.

THE CLOSE RELATION BETWEEN TYPHOID AND PARATYPHOID BACILLI.

There are a large number of articles in the literature on the group agglutination of the different members of the typhoid-colon group of bacilli, all tending to show that the members are more or less closely related to each other. Agglutination of paratyphoid bacilli by the serum of typhoid patients and also the converse phenomenon have been reported by Schottmüller, Drigalski, Schultz, Kayser, Seiffert, Lentz, Kutcher, Grünberg and Rolly, and Korte and Steinberg.[2]

[1] *Arb. a. d. kais. Gesundh.*, 1907, 25, p. 164.

[2] A full review of the literature is to be found in Kutcher's article in Kolle and Wasserman's *Handbuch*, Ergbd. 1, 1907, p. 188.

Some observers have found that the serum in question agglutinated the allied bacillus more quickly than the homologous bacillus (Lentz) or in higher dilution. Grünberg and Rolly found that 70 per cent of typhoid patients agglutinated paratyphoid bacilli; 35 per cent of them in higher dilution than typhoid bacilli; 15 per cent agglutinated colon bacilli also. Drigalski had 26 among 275 typhoid patients whose serum agglutinated paratyphoid bacilli more strongly than typhoid. Bieberstein found that in 5 of his 18 typhoid patients the serum agglutinated colon bacilli in higher dilution than typhoid bacilli.

As for the opsonins, according to the few reports that have appeared, the same group action exists as in the case of agglutinins. Schottmüller and Much[1] found the index of typhoid patients high for paratyphoid as well as for typhoid bacilli, and Clark and Simonds[2] found that the index might be even higher for the former than for the latter.

Such results are explained in one of two ways: either on the ground of a mixt infection resulting in the formation of different agglutinins, or on the ground of related agglutinins, the bacilli of this large group being supposed to cause the formation of antibodies which affect to a certain extent the other members of the group. Gaetgens lays great stress on the frequency of mixt infection, especially in typhoid fever, and describes three ways in which this may take place: first, an actual double infection at the outset; second, the entrance of paratyphoid bacilli as saprophytes during the course of typhoid fever; and third, the late appearance and gradual increase of paratyphoid bacilli during convalescence from typhoid fever. That those cases which come under the first and third heads are really mixt infections can be shown by the absorption test. In those cases where paratyphoid bacilli appear during convalescence from typhoid fever, a prolonged observation shows that as the typhoid bacilli disappear the paratyphoid increase, suggesting either that the former are being displaced by an antagonistic organism or that the typhoid bacillus is undergoing a modification. Gaetgens discusses the possibility that paratyphoid bacilli may represent a modified form of typhoid bacilli but rejects the theory as unproven. Still

[1] *Münch. med. Wchnschr.*, 1908, 55, p. 435.

[2] *Ibid.*, p. 496.

there are facts which point to a specially close relationship between the two. For instance, he found in his typhoid convalescents that altho the paratyphoid bacilli appeared in all cases, usually about the sixth week, their appearance was not accompanied by any clinical symptoms, a fact which seemed to indicate that the antibodies already formed against the typhoid bacillus were active against paratyphoid also. He then cites instances of temporary paratyphoid carriers who were at the time in contact with cases of typhoid fever, not paratyphoid. Two chronic paratyphoid carriers with cholelithiasis were carefully examined for the presence of typhoid bacilli but with negative results, yet there was a strong suspicion that they were responsible for the cases of typhoid fever in their environment. Another carrier had a history of typhoid fever at six years of age. At the time of examination her two children had typhoid fever, but her stools showed only paratyphoid bacilli.

Kayser's observations are quoted by Gaetgens[1] in confirmation of his own. Kayser emphasizes especially the importance of paratyphoid bacillus type A as a factor in the mixt infection in typhoid fever. This bacillus may be agglutinated earlier and more completely than the typhoid bacillus in cases of clinical typhoid fever. It may even be grown in pure culture in such cases and agglutination tests prove it to be the pathogenic agent (Blumenthal[2]). Kayser does not consider the agglutinins for typhoid bacilli and paratyphoid bacillus A really specific. He, like Gaetgens, has noted the appearance of paratyphoid bacilli in convalescence from typhoid fever as has also Seiffert.[3]

Among my seven carriers there were three who seemed to illustrate this close relationship between the members of the typhoid-colon group. Case 1 had a history of undoubted typhoid fever four years previously, during which attack typhoid bacilli were isolated from her blood. Her cholecystitis was a sequence of this illness. At the time of my first examination, during the first week after her operation, bile, stools, and urine contained large numbers of paratyphoid bacilli but a most careful search failed to reveal any colonies of typhoid

[1] *Deut. med. Wchnschr.*, 1904, 30, p. 1803.

[2] *Münch. med. Wchnschr.*, 1904, 51, p. 1641.

[3] *Ztschr. f. Hyg.*, 1909, 63, p. 272. See also the epidemic of paratyphoid (B) fever following infection of food by a typhoid carrier described by Fornet, *Arb. a. d. kais. Gesundh.*, 1907, 25, p. 247.

bacillus. Yet at this time the patient's serum gave the following agglutinations: Typhoid bacillus 1:100, paratyphoid bacillus B 1:200. Her opsonic index for the former was from 6.0 to 16.0; for the latter 3 to 8. This proof of the presence of antibodies for the typhoid bacillus induced me to continue my search for this organism, but it was not until 17 weeks later, when the woman was in perfect health, that the plates from her stools yielded, among numerous colonies of paratyphoid bacillus B, a very few colonies of typhoid bacillus. A subsequent examination, three months later, gave only paratyphoid bacilli. This woman's serum at the time of the last examination in April (the date of the operation was November 11) agglutinated her own strain of typhoid bacillus and a laboratory strain in dilution of 1:40, and had an opsonic index of 10 to these strains. At the same time the serum agglutinated her own paratyphoid bacillus in dilution of 1:40 and the laboratory strain in 1:100. Her index was 5.0 to the former, 3.0 to the latter. Castellani's absorption test showed that after the removal of the typhoid agglutinins from the serum, the agglutinins for paratyphoid bacilli were still active. In this case, then, we have apparently a confirmation of the statement that a paratyphoid infection may follow and displace a typhoid infection. There can be no doubt that the cholecystitis was due in the first instance to a typhoid inflammation of the gall-bladder, but at the end of four years' time those bacilli had almost disappeared, altho the serum still was rich in antibodies. Their place had been taken by paratyphoid bacilli which in this case could not be regarded as simply saprophytes because of the specific agglutinin and opsonin for these bacilli in the woman's serum.

Case 2 was in some respects similar, but without a history of typhoid fever. She entered the hospital with the history of frequent attacks of colic, chill, fever, and jaundice. Her blood agglutinated typhoid bacilli in dilution of 1:70; and paratyphoid in dilution of 1:40 only. Her index to the former was 9.8 and to the latter 7.5. No typhoid bacilli could be found in urine or stools at that time.[1] An acute attack of fever and jaundice was followed by a fall in her opsonic index for typhoid bacillus and paratyphoid B (see Chart 1) and as

[1] Most of the typhoid carriers reported in the literature excreted great numbers of these bacilli (see Scheller, *Centralbl. f. Bakt.*, Abt. 1, Orig., 1908, 46, p. 385). Klinger, however, found only very few colonies in the plates from his carriers (*Arb. a. d. kais. Gesundh.*, 1906, 24, p. 91).

she convalesced from this attack the indices rose again. A second attack had the same effect and it was at this time that a few colonies of typhoid bacilli were found in the plates from the clay-colored stools. Shortly after this the patient was operated on for the removal of gall-stones and the bile at the time of operation yielded an almost pure culture of typhoid bacillus. Her serum agglutinated her own strain of typhoid bacilli in dilution of 1:100 and her index at two observations was 3.0 and 7.0. There was no reason to suspect this woman of being a typhoid carrier aside from the discovery of antibodies for the typhoid bacillus in her serum. Not only did she deny any history of typhoid fever, but she had never nursed or even been in contact with any case of typhoid fever, and as all the early examinations of her stools were negative she would certainly have escaped detection if the high opsonic index and agglutinative power of her serum had not pointed to the presence of typhoid infection somewhere.

The two following cases were harder to interpret. Case 3 had had typhoid fever 12 years previously and an attack of what was diagnosed as paratyphoid fever 4 months before. She was in perfect health at the time of the examination and her serum did not agglutinate either typhoid or paratyphoid bacilli to any significant extent (B. typhosus 1:30) but did show an index of 5.2 to 21.0 for typhoid bacilli and of 3.3 to 15.0 for paratyphoid B. Only three specimens of stools could be obtained, from two of which a paratyphoid bacillus type B was obtained, but no typhoid colonies could be found.

Case 8 was not a carrier but was suffering from an attack of paratyphoid fever. She was a trained nurse and had been frequently in contact with typhoid patients but had never had typhoid fever. Her stools contained enormous numbers of paratyphoid bacilli which were agglutinated by her serum in dilution of 1:150 and her opsonic index ran between 1.9 and 14.0. No typhoid bacilli were ever found in the stools, yet her index to this organism was always high, between 4.0 and 22.0, and her serum agglutinated it in dilution of 1:40.

It might be said that in these last two cases we have to do with group agglutinins and group opsonins, as no mixt infection was proven, but it is at least possible that these patients were formerly typhoid carriers and that the gradual substitution of paratyphoid for typhoid

bacilli was so nearly complete as to make detection of these last impossible, though the production of antibodies still continued. If the high opsonic index in these cases and the presence of agglutinin for typhoid bacilli were to be regarded as due only to the group action of the antibodies formed in paratyphoid infection, then the same features should have been encountered in Cases 5, 6, and 7, all of whom, however, had normal indices to typhoid bacilli and no agglutinin, at the same time that they had high indices and agglutinin for paratyphoid bacilli. Case 4 lies between the two, and might be explained as an instance of group action, for while the serum agglutinated typhoid bacilli in dilution of 1:50, the opsonin for the typhoid bacillus was never very much increased. It is worth noting that there was no increase of opsonin for paratyphoid type A in the serum of carriers of type B. For instance Case 3 had an index of 12.0 to type B and of 1.2 to type A. Case 9 had an index of 5.2 to type B and a normal index to type A.

Bieberstein's work on group agglutinins for typhoid bacilli and colon bacilli was referred to above. No instance of agglutination of stock strains of colon bacilli was found in my cases, but, on the other hand, there were three cases who had both agglutinin and high opsonic index to their own strain of colon bacilli, namely Cases 1, 2, and 8. The sera of these patients agglutinated their own strains in dilutions of 1:50 to 1:150 and their opsonic indices rose to 4.6, 6.8, and 10.0 respectively. In Cases 1 and 2 Castellani's absorption test showed that the agglutinins for the homologous strains of colon bacilli were not removed by the removal of the agglutinin for typhoid bacilli, so that here we have to do with real mixt infection.[1]

SUMMARY.

Among 24 cases of chronic cholecystitis, five paratyphoid carriers were found, one typhoid carrier, and one with both organisms.

Five, or 71 per cent, agglutinated their own bacilli or stock strains

[1] I was inclined to regard these organisms as nearer to the paratyphoid group than to the colon group, for the serum of all these patients was quite normal to stock strains of colon bacilli while their opsonin and agglutinin for their own strains corresponded to their opsonin and agglutinin for paratyphoid bacillus B. Culturally the bacilli differed from typical colon bacilli in their growth on gelatin, malachite green agar, Endo plates, and in the fact that they agglutinated with the serum of rabbits immunized against paratyphoid bacillus type B, which serum failed to agglutinate stock strains of colon bacillus.

or both, in dilution of 1:50 or higher. No non-carrier agglutinated any strain in dilution as high as 1:50.

All seven had an abnormal opsonic index to their own bacilli or to some stock strain or to both. In cases with acute symptoms the index fluctuated, falling below normal at times and again rising very high, while in cases free from acute symptoms the index was persistently high, never falling to normal.

Where mixt infection by two organisms existed, agglutination and an abnormal opsonic index for both organisms were found. In one carrier, and in one case of paratyphoid fever, mixt infection was suspected because of the high opsonic index to the two organisms but it could not be proven.

The opsonic index is a very valuable aid in the discovery of bacillus carriers. No decidedly abnormal index was found in any of the non-carriers.

No proof of increased bactericidal power in the serum of carriers was found, nor any proof of increased resistance to bacteriolysis on the part of the carrier's strain. Neither was there any evidence of a greater opsonic power in the carrier's serum toward his own strain, but in three cases the carrier's strain was markedly resistant to phagocytosis.

The statement that a paratyphoid infection frequently follows or accompanies a typhoid infection was apparently confirmed by the study of these carriers.

A close relation exists between the members of the typhoid-colon group, as can be seen by group agglutinins and group opsonins and by the frequent occurrence of mixt infections.